THE FIXATION OF SOLUBLE ANTIGEN BY THE TISSUES.*

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On the injection of an antigen into a susceptible animal a reaction takes place resulting in the formation of antibodies specific for the antigen injected; therefore a study of the seat of fixation of antigen is of importance in the study of antibody formation. Evidence has been presented showing that antibodies are formed (1) locally, that is, by the tissues at the site of inoculation of the antigen; (2) by the blood-forming organs; and (3) by the blood itself, particularly the leukocytes.

Hektoen and Carlson[1] injected dogs intravenously with rat and goat corpuscles—1 c.c. of a 10 per cent suspension per kilo—and three hours to six days after injection tranfused the blood of such dogs into other dogs. They found no antibody formation in the recipients, but if the transfusion took place after the fourth day varying degrees of passive immunity were produced. A perfectly typical antibody production took place in the donors, showing that either sufficient antigen to cause an antibody production in the recipient was not free in the blood of the donor at the time of transfusion or the antigenic power of the injected corpuscles had been destroyed. These results support the view that antibodies are produced outside the blood stream. In these experiments the antigen—goat or rat corpuscles—was injected from three hours to four days before the transfusion. In performing the transfusion the recipient was bled dry, that is, until no more blood flowed from a severed carotid. The blood of the donor—the animal previously injected with antigen—was then pumped into the vessels of the recipient until the blood pressure in the recipient was equal to, or greater than, the pressure in the donor. However, in transfusing from the donor to the recipient until the pressure in both was equalized, it is doubtful if more than half of the blood of the donor

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was transfused into the recipient, especially when most of the recipients used were smaller than the donors. The failure in antibody production in the recipients might be explained by the fact that sufficient antigen-containing blood was not transfused from the donor to recipient.

It is also possible that the rat or goat corpuscles, being morphological units of moderate size, may have been filtered out in the capillaries of the liver and spleen and retained mechanically, especially if agglutination occurred, or the endothelial cells acting phagocytically may have been instrumental in retaining the foreign cells within the body of the donor.

In order to determine more fully whether the retention of antigen in the body of the donor was a mechanical process or a true chemical fixation we decided to repeat the transfusion experiments of Hektoen and Carlson, using as an antigen some soluble substance that could not be filtered out mechanically or taken up phagocytically. To make certain that the major part of the blood was removed from the body of the dog injected with antigen, large recipients and small donors usually were used. The recipients were bled until no more blood flowed from the severed carotid and the blood of the donor was then pumped into the vessels of the recipient. As the recipient was larger than the donor, the donor could be bled until the pulse was scarcely perceptible. After the donor had been bled into the recipient until the pulse in the former was barely palpable a liter or more of normal salt solution was introduced into the vessels of the donor through the jugular or femoral vein. This restored the pulse for a time, "flushed out" the vessels of the donor, and made the exsanguination practically complete. The transfusion was continued until the pulse in the donor was again scarcely perceptible, and then stopped. The carotids of both the donor and recipient were ligated and the donor was restored by infusing normal defibrinated blood into the femoral or jugular vein.

We decided to use egg albumen or goat serum as the soluble antigen and the precipitin test as the indicator of antibody production.

Five dogs of about 10 K. weight were injected intravenously with 2, 4, 8, 20, and 35 c.c. of a 50 per cent solution of egg white. The dog injected with 20 c.c. died seven

days after the injection. Ten days after the injection the serum of the remaining four dogs was tested for precipitin, using egg white in dilution of 1/10, 1/100, and 1/1000 as antigen, and serum dilutions 0.1 to 0.001. All the serums tested were negative in dilution of 0.1 or more. A similar preliminary test was made, using undiluted goat serum as the antigen. One dog was injected intraperitoneally with 30 c.c. undiluted goat serum and three others intravenously with 12, 4, and 2 cc. of serum respectively. After ten days these dogs were tested with the following results:

Dog 1.—30 c.c. intraperitoneally; precipitin test positive to 0.01 c.c.

Dog 2.—12 c.c. intravenously; precipitin test positive to 0.1 c.c.

Dog 3.—4 c.c. intravenously; precipitin test positive to 0.1 c.c.

Dog 4.—2 c.c. intravenously; precipitin test negative in undiluted serum.

Dog 5.—Normal, precipitin test negative in undiluted serum.

Antigen was used in 1/10, 1/100, and 1/1000 dilutions in making the precipitin tests. Antigen in 1/10 dilution gave the best results.

Four other dogs of about 8 to 10 K. weight were injected intravenously with 6, 7, 7, and 8 c.c. goat serum respectively. Ten days later all showed a good precipitin test to 0.1 c.c. using goat serum in 1/10 dilution as antigen.

These findings show that goat serum injected intravenously into dogs in doses of more than 2 c.c. gives a good precipitin reaction in 10 days. After these preliminary tests a series of transfusion

No.	Amount and Place of Injection	Interval between Injection and Transfusion	Days after Transfusion When Test Was Made	Results (Antigen in dilution 1–10)	
				Donors	Recipients
1	8 c.c. intrav.	3½ hours	12	+ (0.1 c.c.)	–
2	8 c.c. intrav.	4½ hours	12	+ (0.1 c.c.)	–
3	8 c.c. intrav.	4¾ hours	12	+ (0.1 c.c.)	–
4	5 c.c. intrav.	3½ hours	9	+ (0.1 c.c.)	–
5	7 c.c. intrav.	3½ hours	9	+ (0.05 c.c.)	–
6	6 c.c. intrav.	3 hours	12	+ (0.25 c.c.)	+ (0.25 c.c.)
7	6 c.c. intrav.	3 hours	12	+ (0.25 c.c.)	–
8	6 c.c. into heart	1 hour	12	+ (0.25 c.c.)	–
9	6 c.c. into heart	1 hour	12	+ (0.25 c.c.)	–
10	6 c.c. into heart	30 minutes	12	+ (0.1 c.c.)	–

+ = Precipitation.

– = No precipitation with undiluted serum.

The figures in parentheses give lowest active dilution of serum.

experiments were conducted to determine whether a soluble antigen was removed from the blood stream under the conditions outlined and the length of time necessary for the removal of a soluble antigen from the blood stream. That is to say, goat serum was injected intravenously into the donor and the blood of the donor transfused into the body of the recipient three, four, one, and one-half hours later in 10 sets of dogs (table on p. 45).

Transfusion technic.—In all cases the carotid of the donor was connected with the external jugular of the recipient using a T cannula of glass. Both donor and recipient were anesthetized with ether and placed on their backs, the left carotid and jugular of the donor and the right carotid and jugular of the recipient were isolated; a cannula was inserted into the proximal end of the jugular of the donor, and one into the proximal end of the carotid of the recipient, and each clamped off with a bulldog forceps. The distal end of the jugular in the donor and the distal end of the carotid in the recipient were ligated.

One arm of the T cannula was then inserted into the proximal end of the carotid of the donor and the distal end of the carotid ligated. The proximal portion of the severed carotid was clamped off with a bulldog forceps. The donor and recipient were then placed neck to neck and the other end of the cross-arm of the T cannula inserted into the proximal end of the jugular of the recipient. The distal end of the jugular in the recipient was ligated. The third arm of the T cannula was filled with salt solution, all air bubbles removed, and this arm clamped off with a bulldog forceps.

The recipient was then bled "dry," that is, until the flow of blood from the carotid ceased. (This blood was gathered in a sterile jar containing glass beads, and defibrinated.) The clamp on the proximal end of the carotid of the donor was then removed and the blood allowed to flow through the cannula from the carotid of the donor into the jugular of the recipient until the pulse in the donor had become scarcely palpable. Salt solution, usually about a liter, was then run into the proximal end of the jugular vein of the donor by gravity. This restored the pulse of the donor for a time and made the exsanguination much more complete than simple bleeding, the salt solution "flushing out" the vessels of the donor. When the blood was much lighter in color (as seen flowing through the glass connecting cannula) and much lowered in viscosity (as tested by allowing some to flow into a vessel from the third arm of the transfusion cannula), and the pulse of the donor was scarcely perceptible, the infusion of salt solution was stopped and the normal defibrinated blood from the recipient infused into the donor. This restored the pulse of the donor almost immediately. When the infusion of defibrinated blood into the donor was under way the connection of donor with recipient was severed, the vessels in the latter ligated, and the wound closed. When most of the defibrinated blood had been infused into the donor the cannulae were taken out of the vessels, the vessels ligated, and the wounds closed.

Small dogs were used as donors and large dogs as recipients. This made it possible to get over more blood, as there was more room in the vessels of the recipient for blood and blood diluted with salt solution. After ten or twelve days the serum of both recipients and donors was examined for precipitins using goat serum in dilution of 1/10 as precipitinogen.

Ten transfusions were made from 30 minutes to four and three-quarters hours after the injection of the soluble antigen—goat serum. The tests for antibody formation were made nine to 12 days after injection and transfusion. The results of this series of experiments are tabulated on p. 45.

An examination of the table shows that in each instance the donor gave a well marked precipitin reaction while the recipients failed to give any reaction in all but one case. This recipient gave a moderate reaction in the undiluted serum and a scant reaction in the 0.5 and 0.25 dilutions. The donor of this set gave strong reactions in the undiluted, 0.5 and 0.25 dilutions. This donor was the smallest one used.

From these experiments it is concluded that mechanical separation or phagocytosis is not a factor in the retention of the foreign corpuscles in the bodies of the donors in the series of transfusions performed by Hektoen and Carlson, and that soluble as well as insoluble antigens are fixed outside of the blood stream.